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Simultaneous determination of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food samples using a carbon composite electrode modified with $Cu_3(PO_4)_2$ immobilized in polyester resin

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ABSTRACT

A simple electrochemical method was developed for the single and simultaneous determination of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food samples using square-wave voltammetry (SWV). A carbon composite electrode modified (MCCE) with copper (II) phosphate immobilized in a polyester resin was proposed. The modified electrode allowed the detection of BHA and BHT at potentials lower than those observed at unmodified electrodes. A separation of about 430 mV between the peak oxidation potentials of BHA and BHT in binary mixtures was obtained. The calibration curves for the simultaneous determination of BHA and BHT demonstrated an excellent linear response in the range from 3.4×10^{-7} to 4.1×10^{-5} mol L^{-1} for both compounds. The detection limits for the simultaneous determination of BHA and BHT were 7.2×10^{-8} and 9.3×10^{-8} mol L^{-1}, respectively. In addition, the stability and repeatability of the electrode were determined. The proposed method was successfully applied in the simultaneous determination of BHA and BHT in several food samples, and the results obtained were found to be similar to those obtained using the high performance liquid chromatography method with agreement at 95% confidence level.

1. Introduction

The synthetic phenolic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Fig. 1) are frequently used to prevent the oxidative rancidity of food, pharmaceutical, and other commercial products. Various studies have shown that these synthetic components could enter in the human body through the intake of foods, pharmaceutical products, etc. These additives could have harmful effects on human health. Therefore, the use of them is subject to regulations which define the permitted percentage of these compounds as well as their concentration limits [1]. In Europe, the above-mentioned antioxidants are strictly regulated to use in foodstuffs. The use of BHA is permitted in bouillons, gravies, dehydrated meat, and dehydrated soups individually or combined up to a maximum limit of 200 mg/kg expressed on the fat content of the product. On the other hand, BHT is not permitted in these foods; however, it may be used in fats and oils. In the United States, the use of BHT is permitted and can be used individually or in combination with BHA [2].

Recently, it has been found that these artificial phenolic antioxidants might cause a loss of nutrients, and even produce toxic effects. In several countries, the use of these antioxidants is controlled [3]. Thus, it is important to reliably determine the amounts of these substances in the above-mentioned products.

In practice, a mixture of two or more antioxidants is found to be more effective for use than the use of a single compound. Since these substances are chemically similar, the analysis becomes difficult at traced levels without previous separation [4]. Generally, chromatographic methods are used for the analysis of these compounds [5–12], but even then previous separation or sample clean up is sometimes required resulting in fairly involved complicated procedures. Electrochemical methods have been recently applied for the analysis of these antioxidants. Thus, methods of the determination of BHA and BHT have been studied on the basis of their oxidation processes by the flow injection technique at a glassy carbon electrode [13,14] and modified electrode [15]. Voltammetric studies of the BHA oxidation process were carried out for determining this antioxidant in chewing gum [16] and cereals [17], and BHA and BHT were simultaneously determined in spiked samples of dehydrated potato flakes by square-wave voltammetry (SWV) and differential pulse voltammetry (DPV) on cylindrical carbon fiber microelectrodes having a difference of about 300 mV between their peak potentials [18]. The catalytic-voltammetric determination of BHA and BHT at a carbon paste electrode modified with nickel phthalocyanine was also reported [19].

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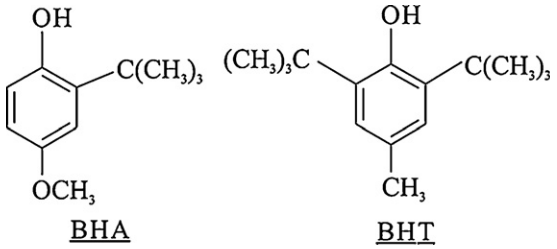

Fig. 1. Chemical structure of BHA and BHT.

Another interesting study for determining the presence of BHA and BHT was developed by Raymundo et al. [20]. In this study, the authors employed square-wave voltammetry (SWV), differential pulse voltammetry (DPV), and cyclic voltammetry in the simultaneous determination of BHA, BHT, and TBHQ, using two working electrodes separately, such as a glass carbon electrode and a platinum electrode. The results obtained using the two electrodes and the different voltammetric techniques were compared considering their sensitivity and detention limits.

Voltammetric techniques offer the possibility of determining the analyte concentration directly in the sample without any special pre-treatment or chemical separation, as well as analyzing colored materials and samples with dispersed solid particles [21].

Square-wave voltammetry (SWV) has proven to be extremely sensitive for organic molecules detection, with a low non-Faradaic current and high sensitivity [22–24]. In contrast to the conventional voltammetric techniques that have detection limits of 10^{-5} mol L^{-1}, the use of SWV technique makes it possible to obtain detection limits of three orders of magnitude lower.

The catalytic action of copper has been reported to be the result of Cu(II)/Cu(I) or at very high positive potential, Cu(III)/Cu(II) redox systems. The oxidation potential of the Cu(II)/Cu(III) redox couple is generally very high and near to the oxidation of both analytes, so the mechanism of analyte(s) oxidation seems unclear [25].

The literature reports the determination of amino acids [26,27] and carbohydrates [28–31] using copper electrode in phosphate and carbonate buffer solutions which made possible the analysis of these organic substances. Currently, the copper electrode has been used in various forms such as metallic form and metallic leagues and also as substrate for film deposits [32,33], modified electrode with oxide [25] and copper phosphate [34].

The method of immobilization of the reagents in polyester resins is much used in flow injection systems to construct solid-phase reactors [35,36]. In addition, it is reported in the literature that copper phosphate immobilized in polyester resin was used in the construction of solid-phase reactor used in FIA for the determination of ascorbic acid in pharmaceutical formulation [36] and aspartame in food samples [37].

The immobilization procedure is fairly expeditious, simple, and presents several advantages. In the present study, the preparation and application as well as the properties of a modified carbon composite electrode containing copper (II) phosphate immobilized in a polyester resin for the determination of BHA and BHT in food samples are described.

2. Experimental

2.1. Apparatus

All voltammetric measurements were carried out in a single-compartment glass cell with a three-electrode system and degassing facilities for bubbling N_2. A carbon composite electrode modified with copper (II) phosphate immobilized in a polyester resin ($Cu_3(PO_4)_2$-Poly) was used as working electrode. Ag/AgCl (3 mol L^{-1} KCl) was used as reference electrode and a platinum wire was used as auxiliary electrode. Square wave and cyclic voltammetric measurements were performed using an AUTOLAB PGSTAT-30 (Ecochemie) potentiostat/galvanostat controlled by the GPES 4.0 software.

The BHA and BHT were determined by high performance liquid chromatography (HPLC) using a method proposed by Saad et al. [8] with some modifications. An LC-10AT Shimadzu system with ultraviolet–vis detector (SPD-M10-AVP) set at a wavelength of 209 nm was used for this purpose. A Shim-Pack CLC-ODS (6.0 mm × 150 mm, 5 μm) chromatographic column was used. Methanol/acetonitrile (50/50%, v/v) at a flow-rate of 1.0 mL min^{-1} was used as the mobile phase, and the injection volume was 30 μL.

2.2. Reagents and solutions

All solutions were prepared using ultra-purified water supplied by a Milli-Q system (Millipore®) with resistivity equal to or higher than 18 MΩ cm. All chemicals used were of analytical reagent grade. Solutions of 0.1 mol L^{-1} phosphate buffer, 0.10 mol L^{-1} sodium nitrate, 0.10 mol L^{-1} potassium nitrate, 0.10 mol L^{-1} sodium chloride, and 0.10 mol L^{-1} potassium chloride (pH range between 2 and 9) were prepared and used in the supporting electrolyte study.

The supporting electrolyte used for all experiments was 0.10 mol L^{-1} potassium nitrate/10% ethanol (pH 6.7; pH was adjusted with 1.0 mol L^{-1} HNO_3). BHA and BHT were purchased from Sigma–Aldrich and a 1.0×10^{-4} mol L^{-1} stock solution was prepared daily in supporting electrolyte/ethanol 90/10% (v/v). The reference solutions were prepared by appropriately diluting the stock solution in supporting electrolyte.

The $Cu_3(PO_4)_{2(S)}$ was prepared by the reaction of $CuCO_3{\cdot}Cu(OH)_2$ (Vetec) with concentrated phosphoric acid (Merck). Immobilization of $Cu_3(PO_4)_2$ was performed using polyester resin (Resapol T-208) and methylethylketone (Ibere, Ramines and Cia.) as a catalyst. Graphite powder (1–2 μm particle size Aldrich) and solid paraffin (Aldrich) were used in the preparation of carbon composite. Mayonnaise samples were obtained from a local supermarket.

2.3. Immobilization of $Cu_3(PO_4)_2$ in polyester resin

Immobilization of $Cu_3(PO_4)_2$ in polyester resin was achieved using a method similar to that proposed by Pereira and Fatibello-Filho [36]. Five grams of polyester resin solution was mixed with 5 g of $Cu_3(PO_4)_{2(S)}$ and 0.3 mL of methylethylketone peroxide (catalyst). After 4 h, a rigid solid was obtained, which was initially broken with the help of a hammer and then ground in a Tecnal multiuse mill, model TE 631/1 (Piracicaba, Brazil). The particles thus obtained were then sieved on a known mesh sieve, and particles smaller than 100 μm were selected for examination.

2.4. MCCE with ($Cu_3(PO_4)_2$-Poly)—preparation and electrochemical measurement

The effects of electrode composition and $Cu_3(PO_4)_2$-Poly percentage were initially studied. In these studies, the graphite: solid paraffin compositions of 75:20, 70:20, 65:20, 60:20, 55:20, 50:20, 45:20, 40:20, and 20:20 (%, m/m), and the $Cu_3(PO_4)_2$-Poly percentages of 5, 10, 15, 20, 25, 30, 35, 40, and 60% (m/m) relating to the total mass of the carbon composite were evaluated. The carbon composite electrode was initially prepared by mixing 0.40 g of ($Cu_3(PO_4)_2$-Poly) (40%, m/m), 0.40 g of graphite powder (40%, m/m), and 0.20 g of melted paraffin (20%, m/m) at 60 °C.

The resulting modified carbon composite was placed in a 1 mL plastic syringe and a copper wire was inserted to obtain the external electric contact. A carbon composite electrode containing 80% (m/m) graphite and 20% (m/m) solid paraffin was prepared for comparison with a modified electrode containing 40% (m/m) $Cu_3(PO_4)_2$-Poly.

Square wave measurements were performed in a 0.10 mol L^{-1} potassium nitrate/10% ethanol (v/v) (pH 6.7 adjusted with HNO_3), performed by applying a sweep potential ranging between 0.0 and 1.2 V.

2.5. Procedure

Subsequent to the optimization of the experimental parameters for the proposed method, the analytical curves were constructed by adding small volumes of standard (reference) solutions of the two analytes in the voltammetric cell. The detection limit was calculated as 3 times the value of the standard deviation of blank solution divided by the slope of the analytical curve.

Three different brands of mayonnaise samples were analyzed using the proposed voltammetric method and the comparative HPLC method. Samples ranging between 10.0 and 20.0 g, accurately weighed to 2 g, were individually dissolved in 5 mL of pure methanol (HPLC methods) or pure ethanol (proposed method) and transferred to a 20 mL centrifuge tube. The mixture was mechanically shaken for 10 min and was submitted to an ultrasonic bath for 30 min at room temperature. Subsequently, the mixture was centrifuged at 3000 rpm for 10 min. The extraction procedure was repeated twice.

All extracts were collected and the solution was then diluted with methanol or ethanol. 1 mL aliquot of this solution was transferred to the electrochemical cell containing 5 mL of 0.1 mol L^{-1} potassium nitrate/10% ethanol; the square-wave voltammograms were obtained subsequent to each aliquot addition.

2.6. Statistics

All experiments were carried out in triplicate to obtain the results. All data were presented as means ± SD. Student's *t*-test at the 95% confidence level was used. $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Electrode composition effect

The effect of the carbon composite composition on the voltammetric profiles of a 2.0×10^{-6} mol L^{-1} BHA in 0.1 mol L^{-1} potassium nitrate/10% ethanol (v/v) solution (pH 6.7) was initially evaluated.

It was found that the amount of $Cu_3(PO_4)_2$ immobilized in polyester resin in the carbon composite presented a significant influence on the voltammetric response of the modified composite electrode. The peak currents increased with increasing amount of chemical modifier up to 40% (m/m). For $Cu_3(PO_4)_2$ amounts higher than 40% (m/m), the peak currents decreased significantly. This occurred owing to a decrease in the graphite powder content in the composite and the consequent reduction of the conductive electrode area. The best carbon composite composition was found for an electrode composition of 40% (m/m) $Cu_3(PO_4)_2$–polyester resin, 40% (m/m) graphite, and 20% (m/m) solid paraffin.

3.2. Investigation of the electrochemical behavior

The electrochemical behavior of BHA and BHT on the MCCE–$Cu_3(PO_4)_2$ was studied by cyclic voltammetry (CV). Various supporting electrolytes for both compounds were investigated, such as phosphate buffer, sodium nitrate, potassium nitrate, sodium chloride, and potassium chloride. The best results were obtained with potassium nitrate/10% ethanol (v/v) at pH 6.7 (pH was adjusted with 1.0 mol L^{-1} HNO_3). The peak oxidation potentials of 440 mV and 870 mV *vs.* Ag/AgCl (3.0 mol L^{-1} KCl) were obtained for BHA and BHT, respectively.

The redox pair peaks corresponding to the electrochemical behavior of the $Cu_3(PO_4)_2$ was observed at 160 mV and 100 mV

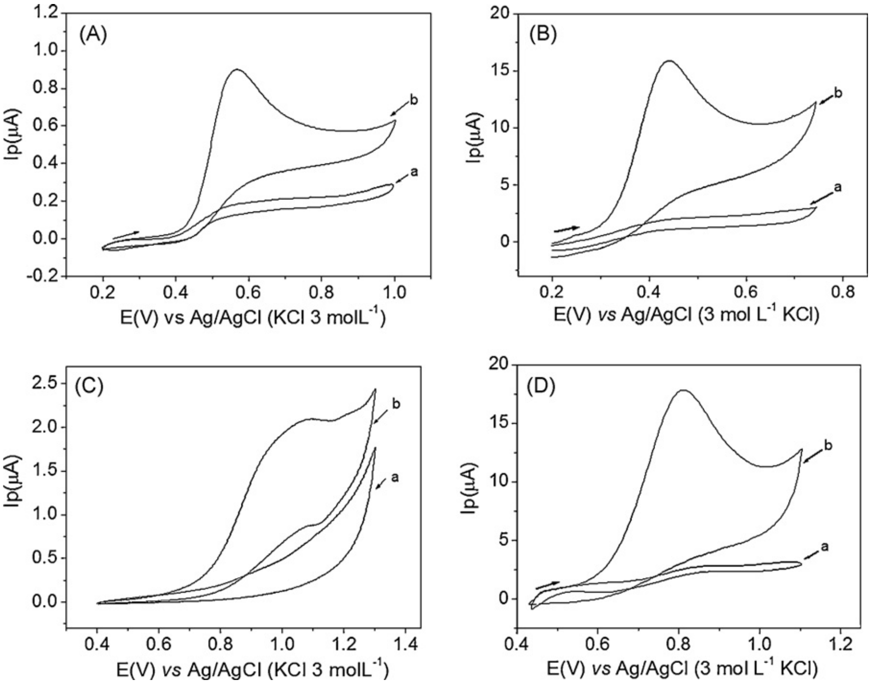

Fig. 2. Cyclic voltammograms acquired (A) for BHA on unmodified electrode in the absence (a) and in the presence (b) of 2.0×10^{-5} mol L^{-1} BHA and (B) on modified electrode (MCCE–$Cu_3(PO_4)_2$-Poly) in the absence (c) and in the presence (d) of 2.0×10^{-5} mol L^{-1} BHA. (C) For BHT on unmodified electrode in the absence (a) and in the presence (b) of 4.0×10^{-5} mol L^{-1} BHT and (D) on modified electrode (MCCE–$Cu_3(PO_4)_2$-Poly) in the absence (c) and in the presence (d) of 4.0×10^{-5} mol L^{-1} BHT. Supporting electrolyte: 0.10 mol L^{-1} KNO_3/10% ethanol (v/v) (pH 6.7); scan rate: 50 mV s^{-1}.

in support electrolyte, corresponding to the anodic and cathodic peaks, respectively. The $Cu_3(PO_4)_2$ modified electrode favored the electrocatalytic oxidation of BHA and BHT to occur at a greatly minimized overpotential for both.

Fig. 2 shows the cyclic voltammograms acquired (A) for BHA using an unmodified carbon composite electrode in the absence (a) and in the presence (b) of 2.0×10^{-5} mol L^{-1} BHA and (B) on modified electrode (MCCE–$Cu_3(PO_4)_2$-Poly) in the absence (c) and in the presence (d) of 2.0×10^{-5} mol L^{-1} BHA. It can be seen from this figure that the current peak obtained with the modified electrode was much higher than that obtained using the unmodified electrode (it is 16 times higher) with a decrease of overpotential of about 145 mV (from 585 mV to 440 mV). In addition, the signal/noise was so improved at the modified electrode. On the other hand, for BHT (Fig. 2C and D) the overpotential decreased from 1080 mV to 870 mV and the analytical signal using the modified composite electrode (MCCE–$Cu_3(PO_4)_2$) was also tremendously improved for a 4.0×10^{-5} mol L^{-1} BHT solution (see details in the legend of Fig. 2), indicating thus the existence of an electrocatalytic process for both analytes. It was also found that the cyclic voltammograms obtained for both BHA and BHT at the MCCE–$Cu_3(PO_4)_2$ presented an irreversible chemical behavior (Fig. 2B and D) due to the absence of a cathodic peak in the backward direction of sweep and the oxidation peak potential shift to more positive potentials with the scan rate increase [38].

The linear plot of the peak current *vs.* the square root of the scan rate (results not shown) was obtained for both, with a 0.998 and 0.999 correlation coefficient for BHA and BHT, respectively. This indicated that the oxidation process of both BHA and BHT was controlled by diffusion [38].

The plot anodic potential *vs.* pH showed a linear relation with the slope of 29.5 and 29.7 mV pH^{-1} for BHA and BHT, respectively (results not shown), which indicated that the BHA and BHT oxidation mechanism involved the transference of two electrons and using the theory developed for the SWV [23,39–42] for irreversible systems (Eq. (1)), the plots peak potential *vs.* logarithms of the square wave frequency was linear (results not shown), which again suggests that BHA and BHT in 0.1 mol L^{-1} KNO_3/10% ethanol (v/v) can be oxidized involving two electrons. However, there is not enough information to propose an electrochemical mechanism of interaction for the oxidation of BHA and BHT at the electrode surface:

$$\frac{\Delta E_p}{\Delta \log f} = -2.3\frac{RT}{\alpha n F} \qquad (1)$$

3.3. Determination of BHA or BHT individually

The effects of the instrumental parameters of SWV (square wave frequency (f), pulse amplitude (a), and scan increment (ΔE_s)) were studied and optimized for the development of electroanalytical procedure. For BHA, the optimization of the experimental parameters was investigated in the presence of 2.0×10^{-5} mol L^{-1} BHA in 0.10 mol L^{-1} potassium nitrate/10% ethanol (v/v) (pH 6.7) and for BHT, these instrumental parameters were investigated in the presence of 4.0×10^{-5} mol L^{-1} BHT in the same electrolyte. The optimum values corresponding to these investigations are shown in Table 1.

The plot of the peak oxidation current as a function of BHA or BHT concentration (results not shown) was linear in the single determination of BHA or BHT in the same concentration range from 3.4×10^{-7} to 4.1×10^{-5} mol L^{-1} for both analytes, with a detection limit (3 times the standard deviation of signal blank/slope) of 5.9×10^{-8} and 6.7×10^{-8} mol L^{-1}, respectively. The linear regression equations are: (ΔIpa/μA) = 2.4×10^{-6} + 0.83 [BHA], r = 0.9989 for BHA and (ΔIpa/μA) = 1.8×10^{-6} + 0.48 [BHT], r = 0.9986 for BHT.

Table 1
Investigated square-wave voltammetry (SWV) parameters and their optimum values for the single determination of BHA or BHT.

Parameters	Studied range	Optimum value
BHA		
SW frequency (f) (Hz)	10–100	80
SW amplitude (a) (mV)	10–100	60
Scan increment (ΔE_s) (mV)	1–6	5
BHT		
SW frequency (f) (Hz)	10–100	80
SW amplitude (a) (mV)	10–100	70
Scan increment (ΔE_s) (mV)	1–6	5

Table 2
Investigated square-wave voltammetry (SWV) parameters and their optimum values for the simultaneous determination of BHA and BHT.

Parameters	Studied range	Optimum value
SW frequency (f) (Hz)	10–80	50
SW amplitude (a) (mV)	10–100	70
Scan increment (ΔE_s) (mV)	1–6	5

3.4. Simultaneous determination of BHA and BHT

The SW voltammograms presented peak oxidation potentials at 440 mV for BHA and at 870 mV for BHT. This good separation of 430 mV of the peak potential clearly allows the simultaneous determination of the compounds. To further investigate the electrochemical response when both substances are present in solution, SWV curves were obtained in the presence of BHA and BHT in 0.1 mol L^{-1} potassium nitrate solution (pH 6.7) containing 10% ethanol (v/v). The SWV optimum parameters are presented in Table 2.

The SW voltammograms were obtained for the different concentrations of BHA with a fixed concentration of BHT (2.0×10^{-5} mol L^{-1} in 0.1 mol L^{-1} KNO_3/10% ethanol (pH 6.7) using the set of optimum conditions described in Table 2 and are shown in Fig. 3. It can be observed that the anodic peak current corresponding to the oxidation process of BHA was linear in the concentration range from 3.4×10^{-7} to 4.1×10^{-5} mol L^{-1} with a detection limit of 7.2×10^{-8} mol L^{-1}. The linear regression equation is: (ΔIpa/μA) = 6.0×10^{-6} + 0.66 [BHA], r = 0.9987) (insert of Fig. 3).

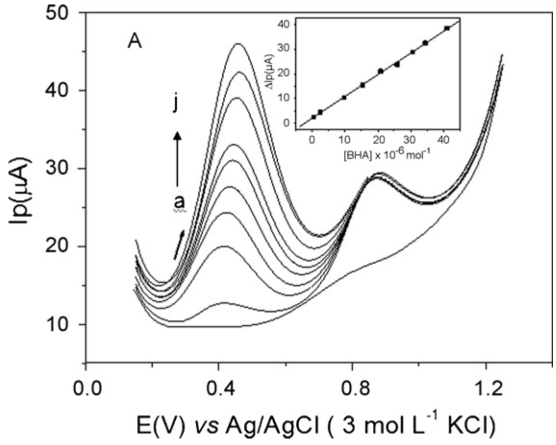

Fig. 3. SW voltammograms (f = 50 Hz; a = 70 mV; ΔE_s = 5 mV) acquired on MCCE–$Cu_3(PO_4)_2$-Poly for various BHA concentrations at a fixed concentration of BHT (2.0×10^{-5} mol L^{-1}). (a) Supporting electrolyte; (b) 3.40×10^{-7} mol L^{-1}; (c) 2.37×10^{-6} mol L^{-1}; (d) 9.62×10^{-6} mol L^{-1}; (e) 1.54×10^{-5} mol L^{-1}; (f) 2.09×10^{-5} mol L^{-1}; (g) 2.59×10^{-5} mol L^{-1}; (h) 3.06×10^{-5} mol L^{-1}; (i) 3.45×10^{-5} mol L^{-1}; (j) 4.12×10^{-5} mol L^{-1}; insert: analytical curve. Supporting electrolyte: 0.1 mol L^{-1} KNO_3/10% ethanol (v/v) (pH 6.7).

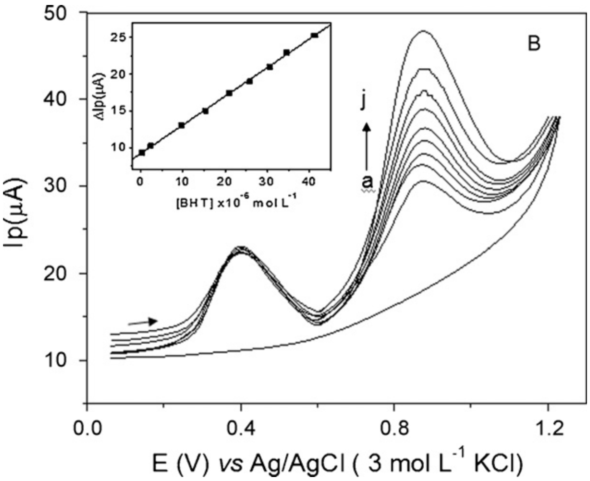

Fig. 4. SW voltammograms ($f = 50$ Hz; $a = 70$ mV; $\Delta E_s = 5$ mV) acquired on MCCE–$Cu_3(PO_4)_2$-Poly for various BHT concentrations at a fixed concentration of BHA (9.0×10^{-6} mol L^{-1}). (a) Supporting electrolyte; (b) 3.40×10^{-7} mol L^{-1}; (c) 2.37×10^{-6} mol L^{-1}; (d) 9.62×10^{-6} mol L^{-1}; (e) 1.54×10^{-5} mol L^{-1}; (f) 2.09×10^{-5} mol L^{-1}; (g) 2.59×10^{-5} mol L^{-1}; (h) 3.06×10^{-5} mol L^{-1}; (i) 3.45×10^{-5} mol L^{-1}; (j) 4.12×10^{-5} mol L^{-1}; insert: analytical curve. Supporting electrolyte: 0.1 mol L^{-1} KNO_3/10% ethanol (v/v) (pH 6.7).

The SW voltammograms were obtained for different concentrations of BHT with a fixed concentration of BHA (9.0×10^{-6} mol L^{-1} in 0.1 mol L^{-1} KNO_3/10% ethanol (pH 6.7)) using the set of optimum conditions described in Table 2 and are shown in Fig. 4; insert of Fig. 4, the analytical curve. It can be observed that the anodic peak current corresponding to the oxidation process of BHT was linear in the concentration large range of 3.4×10^{-7} to 4.1×10^{-5} mol L^{-1} with a detection limit from 9.3×10^{-8} mol L^{-1}. The linear regression equation is: (ΔIpa/μA) = $9.1 \times 10^{-8} + 0.39$ [BHT], $r = 0.9994$.

Based on Fig. 3, it can be concluded that the peak oxidation current for BHA increases linearly with the increasing of BHA concentration at a fixed concentration of BHT (its peak oxidation current remains constant). Similarly, as seen in Fig. 4, the peak oxidation current for BHT increases linearly with an increase in its concentration at a fixed concentration of BHA (its peak oxidation current remains constant).

Subsequent this previous study, BHA and BHT were determined by simultaneously changing their equal concentrations. The SW voltammograms obtained for the solutions containing BHA and BHT in 0.1 mol L^{-1} KNO_3/10% ethanol (pH 6.7) are shown in Fig. 5. The analytical curves for BHA and BHT are shown in Fig. 5B and C, respectively.

The calibration curves for BHA and BHT (Fig. 5B and C) present a good linear response in the concentration range from 3.4×10^{-7} to 4.1×10^{-5} mol L^{-1}. The linear regression equations are: (ΔIpa/μA) = $2.2 \times 10^{-6} + 0.86$ [BHA], $r = 0.9994$ for BHA; and (ΔIpa/μA) = $1.1 \times 10^{-5} + 0.45$ [BHT], $r = 0.9997$ for BHT. The calculated detection limits were 6.8×10^{-8} and 9.1×10^{-8} mol L^{-1}, respectively.

It should be noted that these detection limits are quite low when compared to those obtained by Ni et al. [43], which used a glassy carbon electrode and the chemometric approaches and Kumar and Narayanan [17] using a modified electrode for BHA determination in cereals.

It is important to note that the synergism between BHA and BHT described in some papers [44–46] was not observed in those voltammograms since BHA did not interfere in the determination of BHT and BHT did not interfere in the determination of BHA Thus, the simultaneous determination of BHA and BHT could be carried out with good sensitivity, precision and accuracy.

The repeatability was determined by successive voltammetric measurements ($n = 10$) of the modified carbon composite electrode in 0.10 mol L^{-1} potassium nitrate/10% ethanol (v/v) solution (pH 6.7) containing 2.0×10^{-6} mol L^{-1} BHA and BHT solution; between each voltammetric measurement, the surface of the electrode was not renewed corresponding relative standard deviations (RSD) of 1.2 and 2.3% were obtained, respectively.

The reproducibility was evaluated by measuring the oxidation current values for a period of 5 days. The RSD values obtained, 2.7 and 3.2%, respectively, were observed in the measurements with the fresh solutions prepared daily.

3.5. Interference studies

The effects of some possible interferents were investigated by the addition of compounds to a solution containing 2.0×10^{-5} mol L^{-1} BHA and BHT in 0.1 mol L^{-1} KNO_3/10% ethanol (pH 6.7). The analyses were realized for citric acid, sodium benzoate, starch, potassium sorbate and lactose, because these are commonly used in this kind of product. The different concentrations ratios were tested: 1:1 (interferent:analyte), 1:10, and

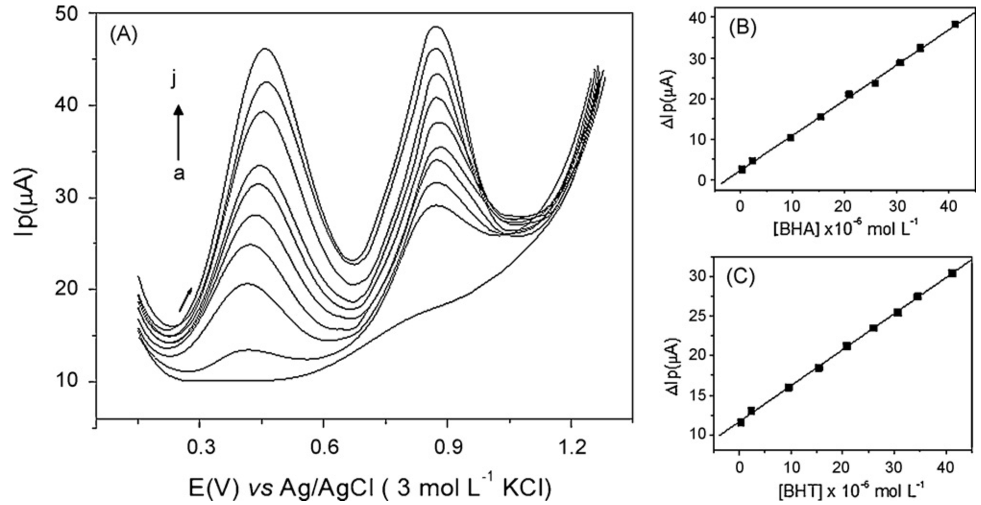

Fig. 5. Simultaneously SW voltammograms ($f = 50$ Hz; $a = 70$ mV; $\Delta E_s = 5$ mV) acquired on MCCE–$Cu_3(PO_4)_2$-Poly for BHA and BHT (A) in 0.1 mol L^{-1} KNO_3/10% ethanol (v/v) (pH 6.7). (a) Supporting electrolyte; (b) 3.40×10^{-7} mol L^{-1}; (c) 2.37×10^{-6} mol L^{-1}; (d) 9.62×10^{-6} mol L^{-1}; (e) 1.54×10^{-5} mol L^{-1}; (f) 2.09×10^{-5} mol L^{-1}; (g) 2.59×10^{-5} mol L^{-1}; (h) 3.06×10^{-5} mol L^{-1}; (i) 3.45×10^{-5} mol L^{-1}; (j) 4.12×10^{-5} mol L^{-1}. Analytical curves for BHA (B) and BHT (C).

Table 3
Results obtained in the simultaneous determination of BHA and BHT in food samples by HPLC and the proposed method (SWV).

Samples	HPLC (mg/100 mL)	SWV (mg/100 mL)	*Re* (%)
A			
BHA	2.40 ± 0.04	2.50 ± 0.08	+4.2
BHT	3.12 ± 0.05	3.07 ± 0.06	−1.6
B			
BHA	5.96 ± 0.04	5.87 ± 0.05	−1.5
BHT	3.37 ± 0.05	3.32 ± 0.08	−1.5
C			
BHA	1.60 ± 0.04	1.59 ± 0.07	−0.62
BHT	1.04 ± 0.03	1.10 ± 0.07	+5.8

Average of 3 measurements. *Re* (%), relative error % = 100 × (voltammetric value − HPLC value)/HPLC value.

10:1 of each interferent cited. The results were compared with those obtained using BHA and BHT standard solution. The obtained responses were analyzed and it was concluded that these compounds do not significantly interfere in the quantification of BHA and BHT by the proposed method.

A recovery study was performed using three brands of mayonnaise samples with three different standard concentrations: 1.45, 4.27, and 7.00 mg L^{-1} of BHA and BHT and the results obtained were compared with those added. The results obtained show that average recovery varied from 96.2 to 102% for the simultaneous determination of BHA and BHT, which indicated that there were no important matrix interferences for the samples analyzed by the proposed method, thereby demonstrating the accuracy of the developed proposed electrode.

3.6. Application of the proposed electrode

The proposed method was applied for the simultaneous determination of BHA and BHT in mayonnaise samples. The HPLC procedure proposed by Saad et al. [8] was modified and used as the comparative method.

The results obtained are summarized in Table 3. It can be seen that the value obtained for BHA and BHT agrees with the expected concentration levels for these antioxidants in this kind of sample (Health Department-Brazil) [47]. The SWV technique was found to be favorable for the detection of synthetic antioxidants in the mixture. It was found that both BHA and BHT showed a greater linear range concentration in the course of this study.

A significant advantage of this method is the matrix effect reduction for complex food samples like mayonnaise.

It can be observed from Table 3 that there are no significant differences between the values found with the SWV and HPLC techniques for the amounts of BHA and BHT in food samples. The statistical calculations for the assay results indicated good accuracy of the method. According to the paired *t*-test, the calculation values ($t_{exp} = 1.51$ for BHA and $t_{exp} = 1.99$ for BHT) were smaller than the label value (t_{label} 2.015). Thus, there are no significant differences between the results obtained by either procedure at the 95% confidence level, which indicates that the carbon composite electrode modified can be used for the simultaneous determination of BHA and BHT in such samples.

4. Conclusions

The obtained results allow concluding that SWV proposed method used a carbon composite electrode modified (MCCE) with $Cu_3(PO_4)_2$ immobilized in polyester resin can be used with some benefits for the quantitative determination of BHA and BHT, single or simultaneous as commonly found in food samples.

For application to these mixtures, the developed method is easier to be adopted and yields lower detection limits than the voltammetric method reported by Ni et al. [43]. Another advantage of the proposed method is the reduced time-consumption and lower cost as compared to the other analytical methods applied for the determination of these substances, especially HPLC.

To conclude, the proposed procedure was effective for the simultaneous determination of BHA and BHT in complex food samples like mayonnaise, without the use of any chemometric approaches or prior treatments. The proposed electrode offers the advantages of reliability, simplicity of the preparation method, good precision, and low cost.

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